VERIFICATION OF TRANSLATION

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[Document Name] [Claims]

[1] A cancer gene therapeutic drug comprising a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell, the carrier cell being selected from the following (1) to (4).

(1) A549 cells

(2) 293 cells

(3) SW626 cells

(4) HT-III cells

[2] The cancer gene therapeutic drug according to claim 1, wherein the oncolytic virus to be infected to the carrier cell has IAI.3B promoter, midkine promoter, β-HCG promoter, SCCA1 promoter, cox-2 promoter, PSA promoter or another tumor specific promoter, according to a kind of cancer to be treated etc.

[3] The cancer gene therapeutic drug according to claim 1 or 2, wherein the oncolytic virus are selected from adenovirus, herpes virus, lentivirus such as HIV virus, retrovirus, reovirus, and any other oncolytic viruses.

[4] A cancer gene therapeutic drug comprising a combination of: a virus for immunological treatment to be administered for inducing a CTL reaction within a living body to administration of a carrier cell; and a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell within the living body.

[5] The cancer gene therapeutic drug according to claim 4, wherein the virus for immunological treatment and the oncolytic virus are selected from adenovirus, herpes virus, lentivirus such as HIV virus, retrovirus, reovirus, and any other oncolytic viruses.

[6] The cancer gene therapeutic drug according to claim 4 or 5, wherein the virus for immunological treatment is a non-proliferative type and/or an inactivated virus.

[7] The cancer gene therapeutic drug according to anyone

of claims 4 to 6, wherein the carrier cell is selected from A549 cell, 293 cell, SW626 cell, HT-III cell, PA-1 cell and any other human derived cancer cell or normal cell.

[8] The cancer gene therapeutic drug according to claim 7, wherein the oncolytic virus to be infected to the carrier cell has IAI.3B promoter, midkine promoter, β-HCG promoter, SCCA1 promoter, cox-2 promoter, PSA promoter or another tumor specific promoter, according to a kind of cancer to be treated etc.

[9] A cancer gene therapeutic method comprising a step for administration of a virus for immunological treatment to induce a CTL reaction within a human body to administration of a carrier cell; and after a predetermined period, a step for at least one administration of a carrier cell to be infected with an oncolytic virus before the administration so as to make the oncolytic virus act on a tumor cell within the human body.

[10] The cancer gene therapeutic method according to claim 9, wherein the period from administration of the virus for immunological treatment to administration of the carrier cell is set about two weeks or more, and not more than 13 weeks.

[11] The cancer gene therapeutic method according to claim 9, wherein the administration rate of the virus for immunological treatment is set in a range from 10^7 viral particles to 10^{11} viral particles.

[12] The cancer gene therapeutic method according to claim 9, wherein one administration rate of the oncolytic virus through the carrier cell is set in a range from 10^{11} viral particles to 10^{13} viral particles.

[13] The cancer gene therapeutic method according to claim 9, wherein the amount of infection of the oncolytic virus to the carrier cell is set in a range from 5 viral

particles/cell to 2,000 viral particles/cell.

[14] A cancer gene therapeutic method comprising administrating an iron preparation and/or a porphyrin compound during a cancer gene therapeutic method using oncolytic virus.

[15] The cancer gene therapeutic method according to claim 14 comprising administrating an iron preparation and/or 5-aminolevulinic acid (ALA).

[Document Name] Specification
[Title of the Invention] CANCER GENE THERAPEUTIC DRUG
[Technical Field to Which the Invention Pertains]
[0001]

This invention relates to a cancer gene therapeutic drug, and a cancer gene therapeutic method using the therapeutic drug.

[Prior Art]
[0002]

Recently, a cancer gene therapy has been focused attention for cancer therapy, and a variety of gene therapies have been proposed and their clinical trials have been carried out. Among them, a clinical trial of a cancer gene therapy using carrier cells was performed by Freeman *et al.* This cancer gene therapy uses ovarian cancer cells PA-1 with a HSV-tk gene by retrovirus as the carrier cells and its clinical trials for ovarian cancer therapy as well as malignant mesothelioma therapy have been carried out (see non-patent literature Nos. 1 and 2 shown later). Culver *et al.* used mouse NIH-3T3 cells as the carrier cells and performed a clinical trial for cerebral tumor (see non-patent literature No. 3 shown later). Its application for human cancer therapy, however, requires human derived cells as the carrier cells.

[0003]

A gene therapy using ovarian cancer cells PA-1 as the carrier cells was also carried out by Coukos *et al.* (see non-patent literature No.4 shown later). This gene therapy constructs an oncolytic virus which specifically proliferates in tumor cells and the virus is infected to the carrier cells (producer cells) and then the infected carrier cells are administered in the tumor site. Herpes simplex 1 (HSV-1) is used as the oncolytic virus. In an animal experiment, their intraperitoneal administration was performed into a nude mouse model with ovarian cancer

transferred to peritoneal cavity (see patent literature Nos. 1 and 2 shown later).

[0004]

Above mentioned ovarian cancer cells PA-1 show high proliferating ability and can be easily manipulated, but they have a drawback of fragility with small cytoplasm. Therefore, introduction of the HSV-tk gene by retrovirus gives little expression of the HSV-tk gene in the tumor site and no satisfactory antitumor effect was obtained against ovarian cancer or malignant mesothelioma in clinical trials of Freeman *et al.*.

[0005]

Application of PA-1 as carrier cells in the cancer gene therapy with the oncolytic virus HSV-1 showed no significant antitumor effect in comparison to that of a therapy with only the oncolytic virus HSV-1. No frequent administrations can be performed in the cancer gene therapy with virus, because of its neutralizing antibody in the blood. Application of PA-1 cells results in little production of virus due to fragile cells. Their disruption before infection to the target tumor cells by cell to cell interaction, and inactivation of the virus with its neutralizing antibody may lead to no significant antitumor effect.

[0006) Furthermore, patient's own cancer cells or fibroblasts are used as the carrier cells in a clinical trial of cellular immunological gene therapy. This procedure requires a long time to get a stable cell line and is difficult to manipulate. In addition, inconstant individual difference exists in introduction of gene and it is difficult to get a stable effect.

[0007]

Non-patent literature No.1: Human Gene Therapy, 6, 927-939, 1995

Non-patent literature No.2: Human Gene Therapy, 9, 2641-

2649, 1998
Non-patent literature No.3: Science, 256, 1550-1552, 1992
Non-patent literature No.4: Clinical Cancer Research, 5, 1523-1537, 1999
Patent literature No. I: International Publication No. 99/45783, pamphlet
Patent literature No.2: International Publication No. 01/23004, pamphlet

[Disclosure of Invention]
[Problem to be Solved by the Invention]
[0008]

The first purpose of the present invention is to solve the above problems and to find new carrier cells exhibiting potent antitumor effect, particularly in the cancer gene therapy using the oncolytic virus.

[0009]

The second purpose of the present invention is to establish a new cancer gene therapeutic method exhibiting a very potent antitumor effect, and to provide a new cancer gene therapeutic drug used for the therapeutic method.

[Means for solving the problems]
[0010]

The inventors of the present invention have investigated for solving the above problems and found those such as (1) more potent antitumor effect can be obtained by using a specific cell line as the carrier cell in comparison to that of conventional carrier cell and (2) induction and raising of a CTL reaction within a living body through prior administration of a virus for immunological treatment, followed by administration of the carrier cell infected with an oncolytic virus, gives a very potent in *vivo* antitumor effect, and accomplished the present invention.

[0011]

Specifically, the first cancer gene therapeutic drug of the present invention comprises a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell, the carrier cell being selected from the following (1) to (4).

(1) A549 cells

(2) 293 cells

(3) SW626 cells

(4) HT-III cells

[0012]

The oncolytic virus to be infected to the carrier cell preferably has IAI.3B promoter, midkine promoter, β-HCG promoter, SCCA1 promoter, cox-2 promoter, PSA promoter or another tumor specific promoter, according to a kind of cancer to be treated etc. Oncolytic viruses without a tumor specific promoter, such as an E1B gene deficient oncolytic adenovirus of ONYX Pharmaceuticals Inc. and an E1A gene partially deficient type Ad5-Δ24 adenovirus of University of Alabama at Birmingham (UAB), may be used. The oncolytic virus are preferably selected from adenovirus, herpes virus, lentivirus such as HIV virus, retrovirus, reovirus, and any other oncolytic viruses.

[0013]

The second cancer gene therapeutic drug of the present invention comprises a combination of: a virus for immunological treatment to be administered for inducing a CTL reaction within a living body to administration of a carrier cell; and a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell within the living body.

[0014]

The second cancer gene therapeutic drug of the present invention is preferably structured such that (1) the virus for immunological treatment and the oncolytic

virus are selected from adenovirus, herpes virus, lentivirus such as HIV virus, retrovirus, reovirus, and any other oncolytic viruses, (2) the virus for immunological treatment is a non-proliferative type and/or an inactivated virus by ultraviolet etc., (3) the carrier cell is selected from A549 cell, 293 cell, SW626 cell, HT-III cell, PA-1 cell and any other human derived cancer cell or normal cell, and (4) the oncolytic virus to be infected to the carrier cell has IAI.3B promoter, midkine promoter, β-HCG promoter, SCCA1 promoter, cox-2 promoter, PSA promoter or another tumor specific promoter, according to a kind of cancer to be treated etc.

[0015]

The cancer gene therapeutic method of the present invention comprises a step for administration of a virus for immunological treatment to induce a CTL reaction within a human body to administration of a carrier cell; and after a predetermined period, a step for at least one administration of a carrier cell to be infected with an oncolytic virus before the administration so as to make the oncolytic virus act on a tumor cell within the human body.

[0016]

The cancer gene therapeutic method of the present invention is preferably structured such that the period from administration of the virus for immunological treatment to administration of the carrier cell is set about two weeks or more, and not more than 13 weeks, (1) the administration rate of the virus for immunological treatment is set in a range from 10^7 viral particles to 10^{11} viral particles, (2) one administration rate of the oncolytic virus through the carrier cell is set in a range from 10^{11} viral particles to 10^{13} viral particles, (3) the amount of infection of the oncolytic virus to the carrier cell is set in a range from 5 viral particles/cell to

2,000 viral particles/cell (vp/cell, hereinafter).

[Effect of the Invention]

[0017]

As a result of selection and resorting, the first cancer gene therapeutic drug of the present invention uses, as a carrier cell, a cell line such as A549 cells with high antitumor effects both in vitro and in vivo, thereby providing more potent antitumor effect in comparison to those of conventional carrier cells.

[0018]

The second cancer gene therapeutic drug of the present invention is a combination of the two drugs composed of the virus for immunological treatment to be administered in advance and the carrier cell to be administered afterwards. Immunological treatment in advance by administration of the virus such as adenovirus and then administration of the carrier cell having been infected with the oncolytic virus provides a direct antitumor effect by infection of the oncolytic virus to target tumor cells, and further induces the CTL reaction within the living body to the infected target cells providing a very potent in vivo antitumor effect.

[Best Mode for Carrying out the Invention]

[0019]

One embodiment to carry out the present invention will be explained.

[1] The first cancer gene therapeutic drug of the present invention

The first cancer gene therapeutic drug of the present invention comprises a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell, the carrier cell being selected from the following (1) to (4).

(1) A549 cells

(2) 293 cells
(3) SW626 cells
(4) HT-III cells

[0020]

Fig. 1 shows the results of screening of the carrier cells to find out effective carrier cells to use for the cancer gene therapeutic drug. More specifically, the cancer gene therapeutic drug was prepared by infection of the oncolytic virus to candidate cell lines and the results of investigation of cancer cell proliferation inhibitory effects are shown. Adenovirus AdE3-IAI.3B was used as the oncolytic virus. The adenovirus AdE3-IAI.3B has E1A gene and E3 gene, and an ovarian cancer specific IAI.3B promoter (IAI.3B promoter) as a tumor specific promoter at the upper stream of E1A gene. The adenovirus AdE3-IAI.3B was infected to various candidate cell lines at a rate of 500 vp/cell for two days and added to the ovarian cancer cells HEY on culture day two, and the proliferation inhibitory effect on the cancer cells HEY was investigated on culture day five.

[0021]

The vertical axis of Fig. 1 shows cell number capable to obtain 50% proliferation inhibitory effect (IC50) for various candidate cell lines. The less number of cells shows the higher proliferation inhibitory effect. As shown by the Figure, the present investigated cancer cell lines showed high proliferation inhibitory effect in the order of 293 cells, A549 cells, SW626 cells and HT-III cells. The 293 cells, A549 cells and SW626 cells exhibited about 100-fold higher proliferation inhibitory effect in comparison to that in PA-1 cells which have been used as carrier cells. HT-III cells also showed about similar high proliferation inhibitory effect with that of SW626 cells.

[0022]

In addition, oncolytic adenovirus was infected to

the above mentioned 293 cells, A549 cells, SW626 cells and HT-III cells to prepare the cancer gene therapeutic drugs and their cancer cell proliferation inhibitory effect was investigated in the presence of a sufficient amount of anti-adenovirus neutralizing antibodies [Ab(+)]. As shown in Fig. 4, all cancer gene therapeutic drugs which used above mentioned four cell lines as carrier cells showed potent cancer cell proliferation inhibitory effect. The conventional cancer gene therapeutic drug with virus was considered to have a difficulty in frequent administrations because of production of antibodies. However, the use of above mentioned four cell lines as carrier cells provided potent *in vitro* proliferation inhibitory effect despite of the presence of antibodies.

[0023]

In addition, *in vivo* experiments using a subcutaneous tumor nude mouse model showed potent antitumor effect when above mentioned A549 cells, 293 cells and SW626 cells were used as the carrier cell (see Fig. 5 and Fig. 6). The details of these experiments will be explained in the examples described later.

[0024]

As shown above, the cancer gene therapeutic drug obtained by infection of the oncolytic virus to the carrier cell is capable to exhibit high antitumor effect by the use of any one of A549 cells, 293 cells, SW626 cells and HT-III cells as the carrier cell.

[0025]

Above mentioned four cell lines are explained. A549 cells are derived from a non-small-cell lung cancer cell line, and their details are described, for example, in the article of Giard, D.J., Aaronson, S.A., Todaro, G.J., Arnstein, P., Kersey, J.H., Dosik, H., and Parks, W.P., *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J.

Natl. Cancer lnst., 51: 1417-1423, 1973. The 293 cells are derived from human embryonic kidney cells and have been used in many experiments and studies as adenovirus producing cells. The 293 cells are explained, for example, in the article of Xie QW, *et al.*, Complementation analysis of mutants of nitric oxide synthase reveals that the active site requires two hemes. Proc. Natl. Acad. Sci., USA, 93: 4891-4896, 1996. The SW626 cells are metastatic strain of colon cancer in ovary and their details are described, for example, in the article of Fogh J., *et al.*, Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. NatL Cancer lnst., 58: 209-214, 1977. The HT-III cells are uterine cervix squamous ep. cancer cells and their details are described, for example, in the article of Fogh J., *et al.*, Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer lnst., 58: 209-214, 1977. These four cell lines are available from cell preserving organizations such as ATCC (American Type Culture Collection) and other commercially available cells may be used.

[0026]

In the cancer gene therapeutic drug of the present invention, a conventional virus vector used for gene introduction may be used as an oncolytic virus to infect the carrier cell. Adenovirus, adeno-accompanying virus, herpes simplex virus type 1 (HSV-l), herpes simplex virus type 2 (HSV-2), Lentivirus such as HIV virus (AIDS virus), retroviruses such as mouse leukemia virus, reovirus etc. can be exemplified and furthermore other oncolytic viruses may be used. The oncolytic virus is a proliferative virus vector and any virus that modify viral gene so as to specifically proliferate in the target tumor cells or tumor tissues, and fuse or kill target cells with cell lysis (cytolysis) action may be used. For example, adenovirus having E1A or E1B domain necessary for

proliferation may be used.

[0027]

The cancer gene therapeutic drug of the present invention can be applied almost all malignant tumors and may include, for example, ovarian cancer, squamous epithelium cancers (e.g. uterine cervix carcinoma, cutaneous carcinoma, head and neck cancer, esophageal cancer and lung cancer), digestive tract cancers (e.g. colonic cancer, pancreatic cancer, hepatic cancer and gastric cancer), neuroblastoma, cerebral tumor, mammary cancer, testicular cancer and prostatic cancer. In addition, adoption of adenoviruses types 34 and 35 capable of infection to blood cells gives the cancer gene therapeutic drug of the present invention applicable to blood malignant tumors.

[0028]

Types of the tumor specific promoter to be introduced into the oncolytic virus may be selected according to the kind of target cancer. For example, IAI.3B promoter for ovarian cancer, midkine promoter for such as cerebral tumor and malignant glioma, β-HCG promoter for testicular cancer, SCCA1 promoter and SCCA2 promoter for squamous epithelium cancers, CEA promoter for colonic cancer, PSA promoter for prostatic cancer and AFP promoter for hepatic cancer may be used. Naturally, other known tumor specific promoters such as cox-2 promoter having a wide action spectrum and exhibiting promoter activity to various malignant tumors and other cancer specific promoters such as osteocarcine promoter may be used. Above mentioned midkine promoter may be used to various malignant tumors in addition to cerebral tumor and malignant glioma and has wide action spectrum as well as cox-2 promoter.

[0029]

No specific limit is given for the length of each

promoter sequence as far as it exhibits the tumor specific promoter activity. Above mentioned IAI.3B promoter can be designed and prepared according to the disclosures in the pamphlet of International Publication No. 03/025190 and the literature, Cancer Research 63, 2506-2512, 2003 and can be inserted in a virus genome. Above mentioned midlkine promoter, β-HCG promoter and SCCA1 promoter can be designed, prepared and inserted into virus genome according to the disclosures in the pamphlets of International Publication Nos. 02/10368, 01/90344 and 00/60068, respectively.

[0030]

Above mentioned SCCA1 promoter is explained in detail in the article Biochimica et Biophysica Acta, 91522 (2001) 1-8, Molecular cloning of human squamous cell carcinoma antigen 1 gene and characterization of its promoter, Katsuyuki Hamada, Hiroto Shinomiya, Yoshihiro Asano, Toshimasa Kihana, Mari Iwamoto, Yasushi Hanakawa, Koji Hashimoto, Susumu Hirose, Satoru Kyo and Masaharu Ito.

[0031]

For example, preparation of an oncolytic adenovirus can be accomplished by insertion of a tumor specific promoter at the upper stream of a primary gene E1A or E1B essential for the proliferation of adenovirus, or replacement with a primary gene E1A or E1B promoter. Similar insertion of the tumor specific promoter at the upper stream of a gene essential for the proliferation of virus, or similar replacement of the promoter of the gene is performed when viruses other than adenovirus such as HSV-1, HSV-2, retrovirus, and reovirus and are used for the construction.

[0032]

However, it is not necessary for the oncolytic virus to have the tumor specific promoter as far as it has specific proliferative property in the target tumor cells

or tumor tissues. For example, oncolytic adenoviruses such as an El B gene deficient type oncolytic adenovirus of ONYX Pharmaceuticals Inc. or an E1A gene partially deficient type Ad5-Δ24 adenovirus of University of Alabama at Birmingham (UAB) may be used. Thus, oncolytic viruses deficient of a tumor specific promoter may be also used.

[0033]

Infection of the oncolytic virus to the carrier cell can be performed by conventional methods without restriction, for example, seeding of carrier cells on a plate, addition of the oncolytic virus at an amount sufficient to infect all cells, cultivation in RPMI medium and fetal calf serum (FCS) (-), under 95% O_2 and 5% CO_2 atmosphere at 37"C for about 6-36 hours, which is simple and easily operable. In the examples shown later, A549 cells, SW626 cells and HT-III cells were cultured by this method and infected with the oncolytic virus whereas 293 cells were cultured in DMEM medium and 10% FCS(+) and infected with the oncolytic virus. Fetal calf serum (FCS) is preferably kept under FCS(-) for 6-12 hours infection. Infection for further period is preferably carried out under FCS(-) for 6-12 hours and then FCS is added at a concentration of 10%.

[0034]

The amount and period of oncolytic virus infection to the carrier cell may be suitably selected according to factors such as the volume and kind of tumor to be treated, kind and rate of the carrier cell, kind of used oncolytic virus and administration method of the cancer gene therapeutic drug of the present invention. Examples are, without particular restriction, for about 6-24 hours at about 5-250 vp/cell by intraperitoneal administration and for about 12-24 hours at about 5-50 vp/cell by intratumoral administration in use of A549 cells, for about 6-24 hours at about 250-2,000 vp/cell by

intraperitoneal administration and for about 12-24 hours at about 100-250 vp/cell by intratumoral administration in use of SW626 cells, for about 6-24 hours at about 5-50 vp/cell by intratumoral administration in use of 293 cells. As shown above, the amount and period of infection vary according to the kinds and administration methods of the carrier cells. The above examples set them within about 6-24 hours at about 5-2,000 vp/cell by intraperitoneal administration, and about 12-24 hours at about 5-250 vp/cell by intratumoral administration.

[0035]

The carrier cell is preferably kept without infection of the oncolytic virus before use, for the preparation of the cancer gene therapeutic drug of the present invention by infection of the oncolytic virus to the carrier cells. The storage of carrier cells may be, for example, performed in a liquid nitrogen or at about -150°C. On the other hand, the oncolytic virus may be kept, for example, at about -80°C.

[0036]

Before use, the oncolytic virus is infected to the carrier cell by the aforementioned method and the resultant virus infected carrier cells can be administered as it is or together with a conventional pharmaceutical carrier to a human body (or experimental animals such as mouse and rat). The carrier cell is preferably subjected to radiation exposure before or after infected with the virus. Radiation exposure at doses of 60-80 Gy, 20-40 Gy, 20-40 Gy were performed before the administration of A549 cells, SW626 cells or HT-III cells to nude mouse, respectively, as shown in the later-described examples.

[0037]

The cancer gene therapeutic drug of the present invention may be preferably administered as a parenteral preparation. Administration as a parenteral preparation

may be performed by an *in vivo* or *ex vivo* method. The dosage of *in vivo* administration (in other words, the dosage of virus infected carrier cells) may be adjusted according to the volume and kind of tumor, severity of disease, and patient's age and body weight etc. For example, administration may be performed by intracavitary injections such as intravenous injection, intravenous drip infusion, intratumoral injection and intraperitoneal injection. These injection preparations may be prepared by conventional procedures and general diluents such as a saline solution and a cell culture solution may be used. Furthermore, a bactericide, an antiseptic, a stabilizer, a tonicity agent and an analgesic may be added if necessary. No particular limit is given for the blending quantity of the virus infected carrier cells in these preparations and can be set suitably.

[0038]

Above mentioned virus infected carrier cells, of course, may be administered in several divided doses to patients or in several divided courses with optional sets of administration times and intervals.

[0039]

As shown above, the dosage of virus infected carrier cells can be determined according to the volume and kind of tumor, severity of disease, and patient's age and body weight etc. Generally, the dosage of carrier cells can be set between about 10^7 cells and 10^9 cells for one administration whereas the dosage of oncolytic viruses through the carrier cell can be set between about 10^{11} viral particles and 10^{13} viral particles for one administration.

[0040]

The kind of the carrier cell may be suitably selected according to the kind of cancer to be treated. The carrier cell may be modified by a gene recombinant

technology, for example, an artificial expression of a specific protein on the surface of the carrier cell to make easy the binding with the target tumor cells, or treatment such as infection of Sendai virus to the carrier cell.

[0041]

Furthermore, administration of an iron preparation and/or a porphyrin compound may be used to enhance the viral productivity in the cancer to be treated. Porphyrin compounds such as 5-aminolevulinic acid (ALA), hematoporphyrin and photofirin are exemplified. As iron preparations, ferrous sulfate ($FeSO_4$) and ferrous citrate for oral administration, and chondroitin sulfate iron and sugar containing iron oxides for intravenous administration may be exemplified. Administration method is not limited, although injection preparation or oral preparation is preferable, together with the cancer gene therapeutic drug of the present invention.

[0042]

Practically, administration of an iron (Fe) preparation and/or 5-aminolevulinic acid (ALA) could markedly enhance inhibitory effect of the oncolytic adenovirus AdE3-IAI.3B on cancer cell proliferation (see Example 5 below).

[0043]

The oncolytic virus can infect from the carrier cells to the target cells by cell to cell interaction, specifically proliferates in the tumor cells and exerts cell lysis (cytolysis) action of fusion or killing of the tumor cells. The cancer gene therapy with virus was considered to have a difficulty in frequent administrations because of production of its antibodies, however, the carrier cells directly establish infection to the target tumor cells by cell to cell interaction to make frequent administrations possible and a potent antitumor

effect can be expected.

[0044]

[2] The second cancer gene therapeutic drug of the present invention

The second cancer gene therapeutic drug of the present invention is a combination of: a virus for immunological treatment to be administered for inducing a CTL reaction within a living body to administration of a carrier cell; and a carrier cell having been infected with an oncolytic virus before the administration so as to make the oncolytic virus act on a tumor cell within the living body. In other words, it is a combination of two drugs of a virus for immunological treatment administered in advance and a carrier cell then administered. Administration of the virus for immunological treatment such as adenovirus followed by administration of the carrier cell infected with the oncolytic virus induces and raises the CTL reaction within the living body and can obtain a very potent *in vivo* antitumor effect.

[0045]

The cancer gene therapeutic drug of the present invention showed a dramatic antitumor effect in a practical experiment using a syngenic model mouse with normal immune system. Although the details will be described later, the ovarian cancer cells OVHM were subcutaneously transplanted to (C57black+C3H/He) FI mouse and were locally injected with carrier cells (A549 cells) infected with oncolytic adenovirus having an ovarian cancer specific promoter. Mice immunized in advance with adenovirus three months before the injection showed a marked antitumor effect 3-4 days after the start of administration and the tumor was completely disappeared after nine days and lymph node metastasis was also diminished (see Fig. 7).

[0046]

As mentioned above, more potent and dramatic antitumor effect was obtained in the experiment using the mice with normal immune system, producing antibodies. This result shows that the CTL reaction (cytotoxic activity through cytotoxic T lymphocytes) was induced and raised within the living body, by prior administration of the virus for immunological treatment. The conventional cancer gene therapeutic drug with virus was considered to have a difficulty in frequent administrations because of production of its antibodies, however, the cancer gene therapeutic drug of the present invention rather makes use of the immune system within the living body and uses it to attack the virus infected target tumor cells.

[0047]

The virus for immunological treatment is preferably the same kind as the oncolytic virus. Non-proliferative type and/or inactivated virus may be preferably used as the virus for immunological treatment. Non-proliferative type virus inactivated by treatment such as UV irradiation to disrupt DNA may be more preferably used. For example, the adenovirus with EI domain deletion and/or inactivated by UV irradiation to disrupt DNA is favorably used as the virus for immunological treatment.

[0048]

The kind of oncolytic virus or carrier cell, the way of infection are same as those for the first cancer gene therapeutic drug of the present invention. Adoption of above mentioned four cell lines (that is, A549 cells, 293 cells, SW626 cells and HT-III cells) as the carrier cell is preferable; however, cells utilizable as the carrier cell are not limited to the above mentioned four lines and other cells such as PA-1 cells (e.g. particularly herpes virus used as an oncolytic virus), fibroblasts, and other human derived cancer cells, normal cells and patient derived cancer cells may be used as the carrier cell.

[0049]

The tumor specific promoter to be infected to the oncolytic virus gene is preferably selected from the above examples (IAI.3B promoter, midkine promoter, β-HCG promoter, SCCA1 promoter, cox-2 promoter, PSA promoter ,CEA promoter etc.) according to the type of cancer; however, promoters are not limited to the above mentioned promoters. However, it is not necessary for the oncolytic virus to have the tumor specific promoter. For example, oncolytic adenoviruses such as an E1B gene deficient type oncolytic adenovirus of ONYX Pharmaceuticals Inc. or an E1A gene partially deficient type Ad5-Δ24 adenovirus of University of Alabama at Birmingham (UAB) may be used.

[0050]

Dosages of the virus, the carrier cell to be infected with the virus, etc. for immunological treatment in the cancer gene therapeutic drug of the present invention are suitably selected according to patient's antibody titer to its virus, volume and kind of tumor, severity of symptoms, and age and body weight of patient. In general, a single dose of the virus for immunological treatment may be set between about 10^8 viral particles and 10^{11} viral particles. The period from administration of the virus for immunological treatment to administration of the carrier cell may be set about two weeks or more, and not more than 3 months. One administration rate of the oncolytic virus through the carrier cell may be set in a range from 10^{11} viral particles to 10^{13} viral particles. The amount of infection of the oncolytic virus to the carrier cell may be set in a range from 5 viral particles/cell to 2,000 viral particles/cell.

[0051]

As with the first cancer gene therapeutic drug of the present invention, the virus infected carrier cells,

of course, may be administered in several divided doses to patients or in several divided courses with optional sets of administration times and intervals.

[0052]

Practical examples of the second cancer gene therapeutic drug of the present invention are such as (1) a combination of the virus for immunological treatment and the carrier cell, (2) a combination of the virus for immunological treatment, the carrier cell and the oncolytic virus for the infection to the carrier cell, (3) a combination with an iron preparation and/or a porphyrin compound added to the above combinations (1) and (2) to enhance the viral productivity, and (4) a combination with necessary compounds for storage, infection and culture, and preparation of medical preparations (e.g. a reagent, a buffer and an enzyme), or vessels (e.g. for reaction, infection and culture, and storage) added to the above combinations (1)-(3).

[0053]

Similarly, practical examples of the first cancer gene therapeutic drug of the present invention are such as (1) a specific carrier cell, (2) a combination of the virus for immunological treatment and the specific carrier cell, (3) a combination with an iron preparation and/or a porphyrin compound added to the above combinations (1) and (2) to enhance the viral productivity, and (4) a combination with necessary compounds for storage, infection and culture, and preparation of medical preparations (e.g. a reagent, a buffer and an enzyme), or vessels (e.g. for reaction, infection and culture, and storage) added to the above combinations (1)-(3).

[Examples]

[0054]

Examples of the present invention are explained

referring the drawings, however, the scope of the present invention is not restricted by these examples.

[0055]

[Example 1: Screening of the carrier cell and antitumor effect in the presence of antibodies]

The following experiments were carried out to screen cancer cell lines which exhibit potent cancer cell proliferation inhibitory effect as the carrier cell.

[0056]

Adenovirus AdE3-IAI.3B was used as the oncolytic virus for the infection to the carrier cell. The adenovirus AdE3-1 AI.3B has El A gene and E3 gene, and an ovarian cancer specific IAI.3B promoter as a tumor specific promoter at the upper stream of ElA gene. The adenovirus AdE3-IAI.3B was infected to various carrier cells at a rate of 500 vp/cell for two days, and then the carrier cells were added to an ovarian cancer cell line HEY on culture day two and their *in vitro* proliferation inhibitory effects were investigated on culture day five.

[0057]

The results of above experiment are shown in Fig. I. The vertical axis of the graph shows cell numbers capable to obtain 50% proliferation inhibitory effect (IC50) for each cell line, and the less cell number shows the higher proliferation inhibitory effect. As shown in the Figure, the cancer cell lines investigated in the present experiment showed high antitumor effect in an order of 293 cells, A549 cells, SW626 cells and HT-III cells. The 293 cells, A549 cells and SW626 cells exhibited about 100-fold higher proliferation inhibitory effects in comparison to PA-1 cells which have been used as carrier cells. HT-III cells also exhibited similar high proliferation inhibitory effect to that of SW626 cells.

[0058]

Then, it was examined how was difference in the

proliferation inhibitory effect between only the oncolytic virus and a combination of the oncolytic virus and the carrier cell, in the presence of antiviral antibodies. As the carrier cell, 293 cell was used and above mentioned adenovirus AdE3-IAI.3B was infected for two days. The resultant adenovirus AdE3-IAI.3B infected 293 cells and their supernatant (AdE3-IAI.3B 293 cell+SUPT) were placed in a 12-well plate in the presence of the anti-adenovirus antibodies. In each well, about 50,000 cells of the ovarian cancer cell line HEY had been cultured from the preceding day. The anti-adenovirus antibodies were prepared by dilution of the antibodies with 600-fold antibody titer to various antibody titers. In the case of only the oncolytic virus, the adenovirus AdE3-IAI.3B was administered in the 12-wellplate at a rate of 1,000vp/cell, in the presence of the anti-adenovirus antibodies. At culture day five, the respective proliferation inhibitory effects on cancer cells (HEY cells) were investigated.

[0059]

The results of the above experiment are shown in Fig. 2. The vertical axis of the graph shows the dilution rate of the anti-adenovirus antibodies at 50% proliferation inhibitory effect (IC50). In other words, 50% proliferation inhibitory effect was obtained for 293 cells even at about 5-fold dilution rate (l20-fold antibody titer) whereas 50% proliferation inhibitory effect was obtained for only the adenovirus at about 600-fold dilution rate (I-fold antibody titer). As shown above, the carrier cell exhibited the proliferation inhibitory effect even under the condition of high antibody titer.

[0060]

Similarly, the proliferation inhibitory effect to HEY cells was investigated in the presence of anti-adenovirus antibodies in the following conditions, (l) adenovirus infected 293 cell and its supernatant (AdE3-

IAI3B 293 cell+SUPT), (2) a cell supernatant containing adenovirus (AdE3-IAI3B, SUPT), (3) a filtered one with a filter of 0.2μm of the cell supernatant containing adenovirus (AdE3-IAI3B, SUPT, filter) and (4) only the adenovirus (AdE3-IAI3B). The results are shown in Fig. 3. The vertical axis of the graph shows a dilution rate of the anti-adenovirus antibodies at 50% proliferation inhibitory rate (IC50). As shown in the Fig., more potent antitumor effect was obtained in comparison to the other conditions when the carrier cell (293 cell) was used.

[0061]

About carrier cells of 293 cells, A549 cells, SW626 cells and HT-III cells, each proliferation inhibitory effect on the cancer cells HEY was investigated in the presence [Ab(+)] or absence [Ab(-)] of the anti-adenovirus antibodies of 100μl/well. The results are shown in Fig. 4. The vertical axis of the graph shows the number of the cancer cells on culture day five. As shown in the Figure, the most potent proliferation inhibitory effect was obtained when A549 cells were used as carrier cells in four kinds of cells. That is, administration of adenovirus infected A549 cells in the presence of a sufficient amount of anti-adenovirus neutralizing antibodies almost completely inhibited the proliferation of the target cancer cells despite of the presence of the antibodies. Other three kinds of cells also showed sufficient proliferation inhibitory effect in the presence of the antibodies.

[0062]

The cancer gene therapy with virus was considered to have a difficulty in frequent administrations because of production of antibodies. However, application of the carrier cell established direct infection to the target cancer cells by cell to cell interaction and frequent administrations became available. Furthermore, application

of the above mentioned four kinds of cells as the carrier cell provided potent antitumor effect.

[0063]

[Example 2: *in vivo* antitumor effect in a nude mouse subcutaneous tumor model]

Then, *in vivo* antitumor effect of the cancer gene therapeutic drug of the present invention was investigated using a nude mouse subcutaneous tumor model. In the experiment, human ovarian cancer cells RMG-1 were subcutaneously transplanted to 5-week-old nude mouse. After four weeks, the cancer gene therapeutic drug of the present invention was injected six times into a massive tumor of about 10-15 mm diameter and the change of the tumor volume was observed. The results are graphically shown in Fig. 5. In the graph, black square represents "control" which are the results of six times injection of PBS buffer into the tumor, black round represents "AdE3-IAI.3B" which are the results of administration of 1×10^{10} viral particles per mouse of the adenovirus AdE3-IAI.3B, black triangle shows the results of administration of 1×10^{7} SW626 cells per mouse, infected with the adenovirus AdE3-IAI.3B at 250 vp/cell. Black rhombus shows the results of administration of 1×10^{7} 293 cells per mouse, infected with the adenovirus AdE3-IAI.3B at 25 vp/cell. White square shows the results of administration of 1×10^{7} A549 cells per mouse, infected with the adenovirus AdE3-IAI.3B at 50 vp/cell. As shown in the Figure, 293 cells and A549 cells used as carrier cells showed complete disappearance of the massive tumor with about 10-15 mm diameter 50 days after the administration. SW626 cells showed 98% proliferation inhibitory effect.

[0064]

Similar experiment as shown above was carried out by subcutaneous transplantation of human ovarian cancer cells PA-1 to 5-week-old nude mouse. The results are shown

in Fig. 6. As shown in the Fig., the massive tumor with about 10-15 mm diameter completely disappeared by the use of 293 cells and A549 cells as carrier cells. SW626 cells showed complete disappearance of the tumor in four out of five mice.

[0065]

[Example 3: *in vivo* antitumor effect in subcutaneous tumor model mouse with normal immune system]

Then, *in vivo* antitumor effect of the cancer gene therapeutic drug of the present invention was investigated using (C57black×C3H/He) F1 mouse with normal immune function. In the experiment, each antitumor effect was investigated in the following conditions; (1) the ovarian cancer cells OVHM were subcutaneously transplanted to the syngenic model mouse. After 10 days or more, A549 cells infected with the adenovirus AdE3-IAI.3B at a rate of 50 vp/cell followed by radiation exposure were five times administered into a tumor, (2) a 7-week-old syngenic model mouse was immunized in advance with an adenovirus for immunological treatment. After three months, the ovarian cancer cells OVHM were subcutaneously transplanted in a similar manner with that of (1) and then, after 10 days or more, A549 cells infected with the adenovirus AdE3-IAI.3B at a rate of 250 vp/cell followed by radiation exposure were five times administered into a tumor, and (3) five times administrations of PBS buffer into a tumor as a control.

[0066]

The results of the above experiment are shown in a graph of Fig. 7. In the graph, black square represents "control" which are the results of the above condition (3), black round represents "AD(-)-A549" which are the results of the above condition (1), without administration of the adenovirus for immunological treatment. Black triangle represents "Ad(+)-A549" which are the results of the above

condition (2), with administration of the adenovirus for immunological treatment. Non-proliferative type adenovirus having no EI gene was used for the adenovirus for immunological treatment. More specifically, it was an adenovirus with inserted LacZ gene in the downstream of CMV promoter. As shown by the Fig., the above condition (1), without prior immunization by adenovirus, showed 20% antitumor effect in comparison to the control, while the above condition (2), with prior immunization by adenovirus, showed marked antitumor effect 3-4 days after the start of administration and the tumor was completely diminished after nine days with disappearance of lymph node metastasis. As shown by the example, the potent and dramatic antitumor effect in mice with normal immune system, despite of their antibody production, might be caused by induction and raising of the CTL reaction within the living body due to the administration of the adenovirus for immunological treatment.

[0067]

The oncolytic adenovirus is infected from the carrier cells to the target tumor cells by cell to cell interaction, specifically proliferates in tumor cells and is considered to exert cell lysis (cytolysis) action to fuse and/or kill the target tumor cells. The cancer gene therapeutic drug of the present invention is considered to induce potent CTL reaction within the living body by prior administration of the adenovirus for immunological treatment, which eliminates the oncolytic adenovirus infected target tumor cells and induces complete natural elimination of the adenovirus infected tumor cells.

[0068]

One manner of infection of the adenovirus to the target tumor cell is considered a cell fusion caused by the adenovirus. Fig. 8 shows the result of microscopic observation after overnight culture from the

administration of 10,000 viral particles per cell of the adenovirus inactivated by UV irradiation into a well with A549 cells cultured. As shown by arrow marks in the Fig., administration of the adenovirus caused cell fusion and multinucleated cells were sporadically observed. No such cells were observed for A549 cells without adenovirus administration (see Fig. 9).

[0069]

Predicted infection manners other than cell fusion are a cell adhesion to the target cells of carrier cells, and infection of the adenovirus to the target tumor cells by local burst of adenovirus and the carrier cell fragment including the adenovirus. In any way, proliferation of adenovirus having a tumor specific promoter in the adenovirus infected target tumor cells may lead to presentation of a potent antigen (or, a cancer specific peptide in target tumor cells infected by adenovirus, recognized as an antigen secondarily), and the tumor cells may be eliminated by the CTL reaction.

[0070]

[Example 4: Antitumor effect by the use of a midkine promoter]

Then, antitumor effect by the use of a midkine promoter was investigated. Fig. 10 (a) shows the results of investigation of midkine (MK) mRNA expressions in human surgical samples 1-21 by RT-PCR. As shown by the Fig., excessive expression of the midkine mRNA was observed in glioblastoma and malignant glioma such as anaplastic astrocytoma, and in diffuse astrocytoma. Thus, the excessive expression of midkine is observed in many cancers such as cerebral tumor.

[0071]

Fig. 10 (b) shows the results of investigation of midkine mRNA expression by RT-PCR in four cell lines of malignant gliomas in a similar manner shown above. As

shown by the Fig., no expression was observed in U87MG and excessive expression of the midkine mRNA were observed in U251MG, LN319 and U373MG.

[0072]

Fig. 10 (c) shows the results of investigation of midkine protein expression in above mentioned each cell line by Western blotting analysis. No expression was found in U87MG as well as mRNA. Excessive expression of the midkine protein was observed in U251MG, LN319 and U373MG.

[0073]

Then, a promoter assay of the midkine was performed. In the experiment, activity of two different length midkine promoters (600 bases and 2,300 bases) was compared. Plasmids (pGL3-MK600 and pGL3-MK2300) with inserted a luciferase gene at the downstream of respective promoters were introduced to each above mentioned cell line and their respective luciferase activity was investigated to evaluate the promoter activity. The results shown in Fig. 11 revealed a higher promoter activity in 600 base sequence length than that in 2,300 base sequence length in the malignant glioma cell line.

[0074]

Fig. 12 (a) shows a schematic diagram of the oncolytic (cytolysis type) adenovirus structure having a midkine promoter designed in the present experiment. The midkine promoter having a 600 base sequence or a 2,300 base sequence was introduced at the site of 552 bp.

[0075]

Fig. 12 (b) shows the results of investigation of E1A protein expression in above mentioned each cell line infected with three types of adenoviruses by Western blotting analysis. As shown in the Figure, expression of E1A protein of adenovirus was observed only in midkine expressing U251MG, LN319 and U373, by the infection of adenovirus (AdMK600) having a 600 base length midkine

promoter. Expression of E1A protein was observed in all cells including normal brain cells by wild type adenovirus (AdWild) and no expression of the E1A protein was observed in all cells by control virus AdLacZ.

[0076]

Fig. 13 (a) shows the results of comparative investigation of cancer cell proliferation inhibitory effect by three kinds of adenoviruses. Wild type adenovirus (AdWild) showed potent proliferation inhibitory effect in all cells, whereas adenoviruses (AdMK600 and AdMK2300) having the midkine promoter showed the proliferation inhibitory effect only in midkine expressing U251MG, LN319 and U373MG. These results were well correlated with the results of midkine mRNA expression and promoter activity. The adenovirus AdMK600 showed more potent proliferation inhibitory effect than that of AdMK2300 having 2,300 base sequence length.

[0077]

Fig. 13 (b) shows the results of investigation of adenovirus E3 domain's influence on the proliferation inhibitory effect. As shown in the Figure, AdMK600 having E3 domain exhibited about 10-fold potent proliferation inhibitory effect than that of adenovirus having no E3 domain (AdMK600-ΔE3).

[0078]

Fig. 13 (c) shows the results of investigation of antitumor effect of adenovirus in a nude mouse subcutaneous transplantation model. In the Fig., black rhombus marks represent the results of administration of wild type adenovirus AdWild, black square marks represent the results of administration of adenovirus AdMK600 having a midkine promoter, black triangle marks represent the results of administration of adenovirus AdLacz with inserted LacZ gene, and black round marks represent the results of administration of only PBS buffer. As shown in

the Figure, only wild type adenovirus showed antitumor effect in the U87MG without midkine expression. In the U373MG expressing midkine, AdMK600 as well as AdWild gave complete disappearance of tumor. No marked difference was observed between the control with injected only PBS buffer and that with injected AdLacZ.

[0079]

Furthermore, adenovirus having the above mentioned midkine promoter (Ad-MK600) was infected to the carrier cells, and the cancer gene therapeutic drug of the present invention was prepared, and the antitumor effect of the virus infected carrier cells was compared to that of administration of only Ad-MK600. In the experiment, 293 cells and A549 cells were used as carrier cells. Above mentioned U373MG cells were transplanted to 5-week-old nude mouse to give a 10-15 mm massive tumor after three weeks. The cancer gene therapeutic drug of the present invention or only Ad-MK600 was administered and the tumor volume was compared after four weeks. The results are shown in Fig. 14. In the Fig., "Ad-MK600" shows the results of administration of only Ad-MK600, and each "293" and "A549" show the results of administration of the virus infected carrier cells using 293 cells and A549 cells as carrier cells, respectively. As shown in the Figure, administration of the cancer gene therapeutic drug of the present invention showed complete disappearance of the tumor. Administration of only Ad-MK600 showed almost no difference with that of control.

[0080]

[Example 5: Influences of Fe and ALA on the proliferation inhibitory effect of adenovirus AdE3-IAI.3B]

Ovarian cancer cells HEY were cultured in 12-well plate at a rate of 10,000 cells/well and $FeSO_4$ was added at a concentration of 50μg/ml, 5μg/ml, 0.5μg/ml or 0μg/ml on the following day and the cytolysis type adenovirus

AdE3-IAI.3B was added to all wells. The proliferation inhibitory effect of the adenovirus was evaluated by IC50 after five days. The results are shown in Fig. 15. In the Fig., the vertical axis shows relative rate (vp/cell) of viruses at IC50 in each condition. As shown in the Figure, administration of 50μg/ml of $FeSO_4$ and the adenovirus showed about 20-fold, and administration of 5μg/ml of $FeSO_4$ and the adenovirus showed about 8-fold proliferation inhibitory effect, respectively, to that of only adenovirus administration.

[0081]

Next, the ovarian cancer cell line HEY was cultured in a 12-well plate at a rate of 10,000 cells/well and 5-aminolevulinic acid (ALA) was added at a concentration of 50μg/ml, 5μg/ml, 0.5μg/ml or 0μg/ml on the following day and the cytolysis type adenovirus AdE3-IAI.3B was added to all wells. The proliferation inhibitory effect of the adenovirus was evaluated by IC50 after five days. The results are shown in Fig. 16. In the Fig., the vertical axis shows relative rate (vp/cell) of viruses at IC50 in each condition. As shown in the Fig., administration of 50μg/ml of ALA and the adenovirus showed about 100-fold proliferation inhibitory effect to that of only adenovirus administration.

[0082]

Furthermore, the ovarian cancer cell line HEY was cultured in a 12-well plate at a rate of 10,000 cells/well and $FeSO_4$ was added at a concentration of 50μg/ml, 5μg/ml, 0.5μg/ml or 0μg/ml on the following day. In addition, the cytolysis type adenovirus AdE3-IAI.3B and 50μg/ml of 5-aminolevulinic acid (ALA) were added to each well. Only the adenovirus was added to a control. The proliferation inhibitory effect of the adenovirus was evaluated by IC50 after five days. The results are shown in Fig. 17. In the Figure, the vertical axis shows relative administration

rate (vp/cell) of viruses at IC50 in each condition. As shown in the Fig., concurrent administration of 50μg/ml of $FeSO_4$, 50μg/ml of ALA and the adenovirus showed about 1,000-fold proliferation inhibitory effect to that of only adenovirus administration. Concurrent administration of 5μg/ml of $FeSO_4$, 50μg/ml of ALA and the adenovirus showed about 700-fold proliferation inhibitory effect to that of only adenovirus administration, and concurrent administration of 0.5μg/ml of $FeSO_4$, 50μg/ml of ALA and the adenovirus showed about 200-fold proliferation inhibitory effect to that of only adenovirus administration.

[0083]

As mentioned above, it was found that ALA and Fe markedly enhance the proliferation inhibitory effect of the oncolytic adenovirus AdE3-IAI.3B. ALA and Fe elevate the amount of virus production, because PFU assay revealed increased amount of adenovirus production. That is, ALA and Fe can increase the amount of adenovirus production within the cancer cells and significantly increase the antitumor effect.

[0084]

ALA, is known to be a porphyrin taken up into cancer cells, is metabolized to protoporphyrin IX. protoporphyrin IX has photo-sensitizing effect and it can be utilized for the photodynamic therapy (PDT) of superficial cancer, together with excimer dye laser.

[0085]

Above mentioned protoporphyrin IX binds with Fe to give a heme and forms heme proteins such as cytochrome in cells. The heme proteins are involved in respiratory system in intracellular mitochondria, ATP production and protein synthesis. Thus, the heme proteins are involved in protein synthesis, including production of the adenovirus if the adenovirus infected. Therefore, promotion of the

porphyrin metabolism may lead to the increased adenovirus production.

[0086]

The cancer gene therapeutic drug of the present invention, as well as the cancer gene therapy of the present invention, can further increase the therapeutic effect by concurrent use of Fe and/or porphyrin compounds such as ALA. That is, concurrent use of Fe and/or porphyrin compounds such as ALA enhances antitumor effect, even under an infection suppressive condition in the presence of antibodies, by acceleration of the CTL response caused by the increased adenovirus production in the target cells. Concurrent use of Fe and/or porphyrin compounds can enhance antitumor effect not only in a syngenic mouse model but also in a human body.

[0087]

In the cancer gene therapy using the oncolytic virus, concurrent use of Fe and/or porphyrin compounds such as ALA is expected to enhance the therapeutic effect, even if the carrier cells are not used.

Industrial applicability

[0088]

As described above, the cancer gene therapeutic drug of the present invention can be applied almost all malignant tumors and can be expected to exhibit potent antitumor effect including ovarian cancer, squamous epithelium cancers (e.g. uterine cervix cancer, cutaneous carcinoma, head and neck cancer, esophageal cancer and lung cancer), digestive tract cancers (e.g. colonic cancer, pancreatic cancer, hepatic cancer and gastric cancer), neuroblastoma, cerebral tumor, mammary cancer, testicular cancer and prostatic cancer.

[Brief Description of the Drawing]

[Fig. 1]

The graph shows the results of cancer cell proliferation inhibitory effects when using various cell lines as carrier cells.

[Fig. 2]

The graph shows the results of investigation of changes in cancer cell proliferation inhibitory effect of oncolytic viruses without and together with carrier cells in the presence of antiviral antibodies.

[Fig. 3]

The graph shows the results of investigation of cancer cell proliferation inhibitory effect of oncolytic adenovirus infected carrier cells (such as 293 cells) in the presence of antiviral antibodies.

[Fig. 4]

The graph shows the results of investigation of cancer cell proliferation inhibitory effect using carrier cells of 293 cells, *A549* cells, SW626 cells and HT-III cells in the presence of antiviral antibodies.

[Fig. 5]

The graph shows the results of investigation of *in vivo* antitumor effect of a cancer gene therapeutic drug of the present invention, using a tumor model formed by subcutaneous transplantation of human ovarian cancer cells RMG-1 in a nude mouse.

[Fig. 6]

The graph shows the results of investigation of *in vivo* antitumor effect of a cancer gene therapeutic drug of the present invention, using a tumor model formed by subcutaneous transplantation of human ovarian cancer cells PA-1 in a nude mouse.

[Fig. 7]

The graph shows the results of investigation of *in vivo* antitumor effect of a cancer gene therapeutic drug of the present invention, using a subcutaneouos tumor model

mouse with normal immune system.

[Fig. 8]

The Figure shows a microscopic observation of cell fusion due to administration of adenovirus.

[Fig. 9]

The Figure shows a control microscopic observation of A549 cells without administration of adenovirus.

[Fig. 10]

Graph (a) shows the results of investigation of midkine (MK) mRNA expression by RT-PCR in human surgical samples of 1-21; graph (b) shows the results of investigation of midkine (MK) mRNA expression by RT-PCR of four cell lines of malignant gliomas in a similar manner; and graph (c) shows the results of investigation of midkine (MK) expression in the above each cell line by Western blotting analysis.

[Fig. 11]

The graph shows the results of comparative investigation of a promoter activity in the above each cell line using two different length midkine promoters.

[Fig. 12]

Graph (a) shows a schematic structure of the oncolytic adenovirus, having the midkine promoter, designed in the present invention; and graph (b) shows the results of investigation of E1A protein expression in the above each cell line infected with three kinds of adenoviruses by Western blotting analysis.

[Fig. 13]

Graph (a) shows the results of comparative investigation of cancer cell proliferation inhibitory effect with three kinds of adenoviruses; graph (b) shows the results of investigation of adenovirus E3 domain's influence on the proliferation inhibitory effect; and graph (c) shows the results of investigation of antitumor effect of adenovirus in a nude mouse subcutaneous

transplantation model.

[Fig. 14]

The graph shows the results of antitumor effect of carrier cells infected with oncolytic virus having the midkine promoter, compared with administration of oncolytic virus without carrier cells.

[Fig. 15]

The graph shows the results of investigation of influence of Fe on the proliferation inhibitory effect of adenovirus AdE3-IAI.3B.

[Fig. 16]

The graph shows the results of investigation of influence of ALA on the proliferation inhibitory effect of adenovirus AdE3-IAI.3B.

[Fig. 17]

The graph shows the results of investigation of influence of the coexistence of Fe and ALA on the proliferation inhibitory effect of adenovirus AdE3-IAI.3B.

[Document Name] Abstract

[Abstract]

[Object]

Objects of the subject invention is to find a new carrier cell which exhibits a very strong antitumor effect in the cancer gene therapy with the oncolytic virus, to establish a new cancer gene therapeutic method exhibiting a very potent antitumor effect, and to provide a new cancer gene therapeutic drug used for the therapeutic method.

[Method for Achieving the Object]

The first cancer gene therapeutic drug of the present invention comprises a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell, the carrier cell being selected from A549 cells, 293 cells, SW626 cells and HT-III cells, which are confirmed to have a strong antitumor effect. The second cancer gene therapeutic drug of the present invention comprises a combination of: a virus for immunological treatment to be administered for inducing a CTL reaction within a living body; and a carrier cell to be infected with an oncolytic virus so as to make the oncolytic virus act on a tumor cell within the living body.

[Selected Figure]

None

Fig 1

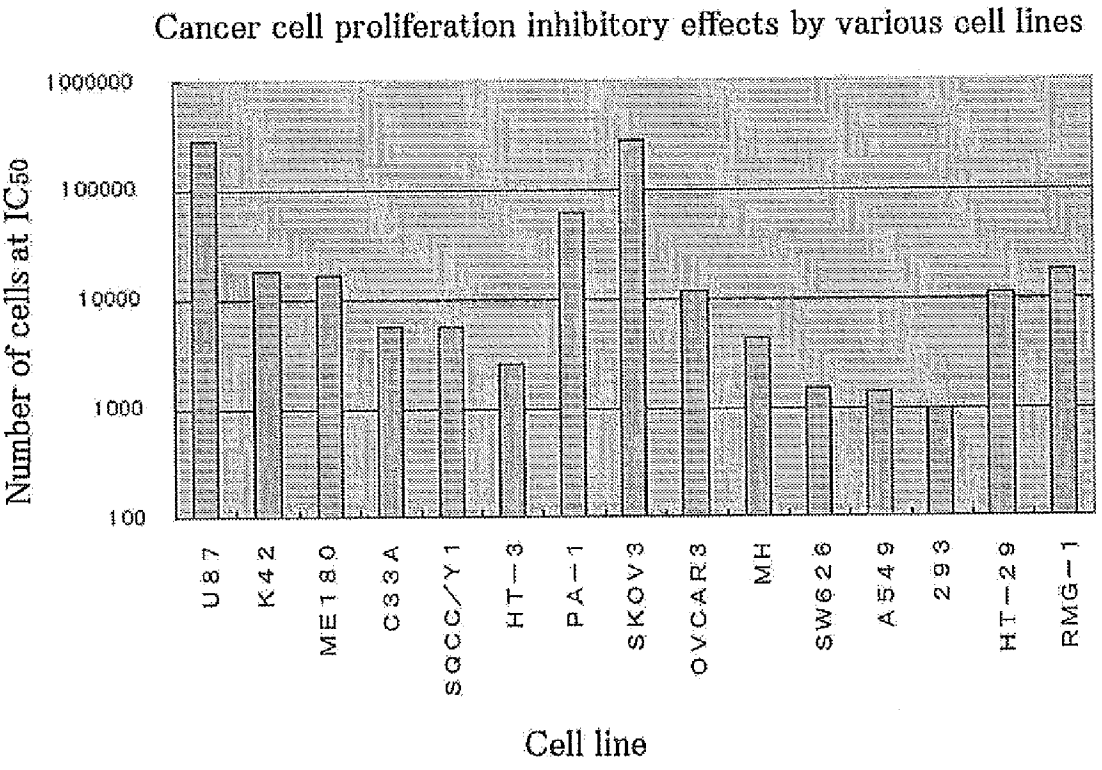

Cancer cell proliferation inhibitory effects by various cell lines
Number of cells at IC50
1000000
100000
10000
1000
100
U87
K42
ME180
C33A
SQCC/Y1
HT-3
PA-1
SKOV3
OVCAR3
MH
SW626
A549
293
HT-29
RMG-1
Cell line

Fig 2

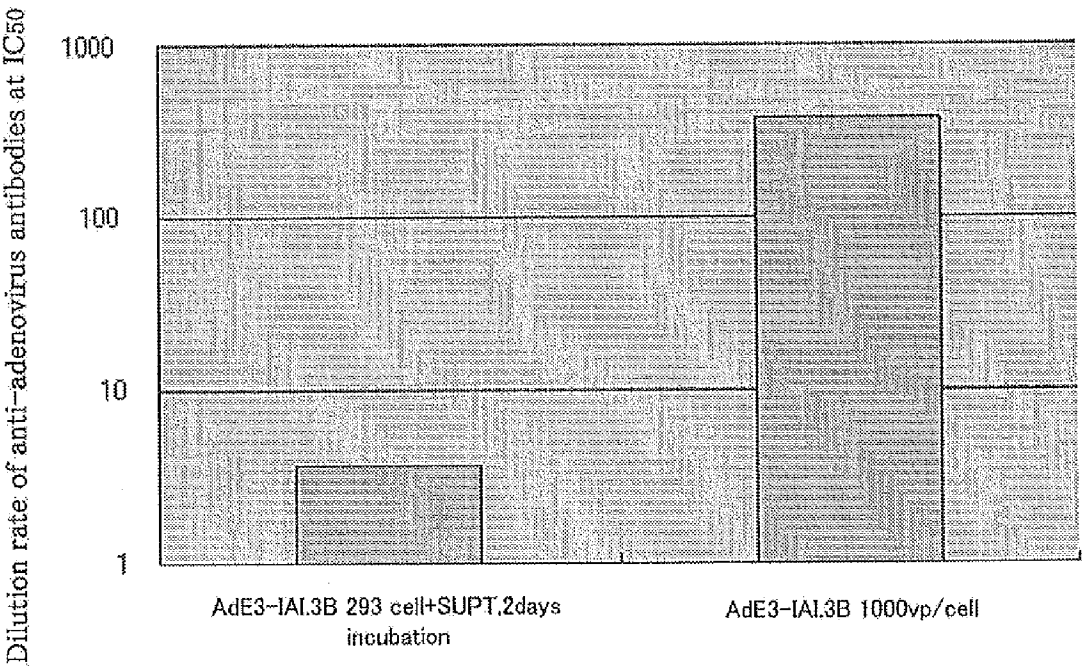

Dilution rate of anti-adenovirus antibodies at IC50
1000
100
10
1
AdE3-IAI.3B 293 cell+SUPT.2days incubation
AdE3-IAI.3B 1000vp/cell

Fig 3

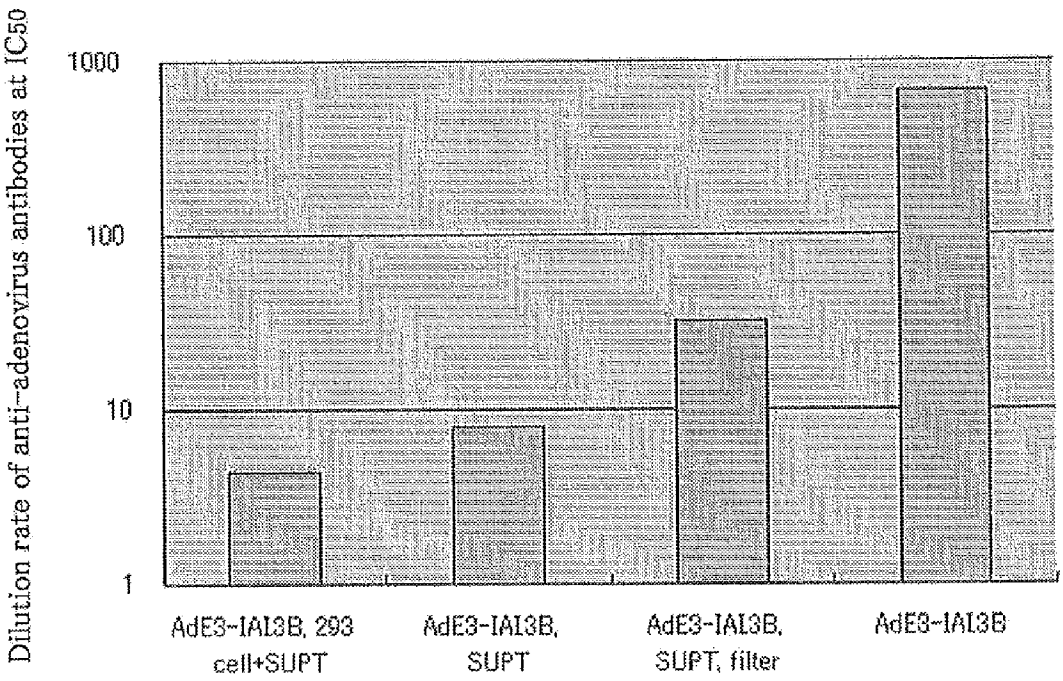

Dilution rate of anti-adenovirus antibodies at IC50
1000
100
10
1
AdE3-IAL3B, 293 cell+SUPT
AdE3-IAL3B, SUPT
AdE3-IAL3B, SUPT, filter
AdE3-IAL3B

Fig 4

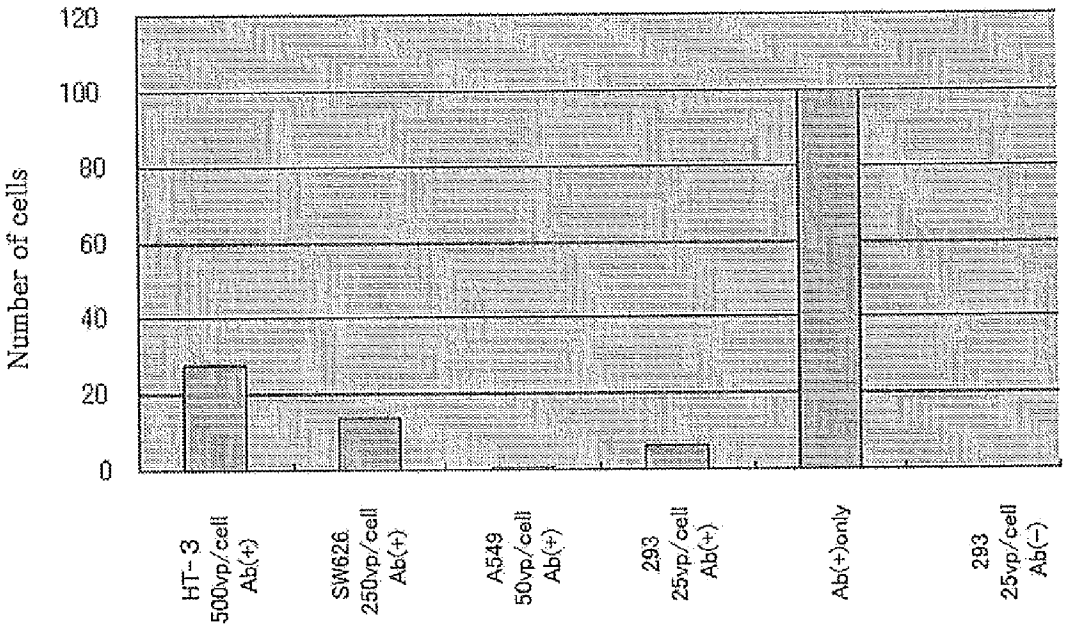

Number of cells
120
100
80
60
40
20
0
HT-3 500vp/cell Ab(+)
SW626 250vp/cell Ab(+)
A549 50vp/cell Ab(+)
293 25vp/cell Ab(+)
Ab(+)only
293 25vp/cell Ab(-)

Fig 5

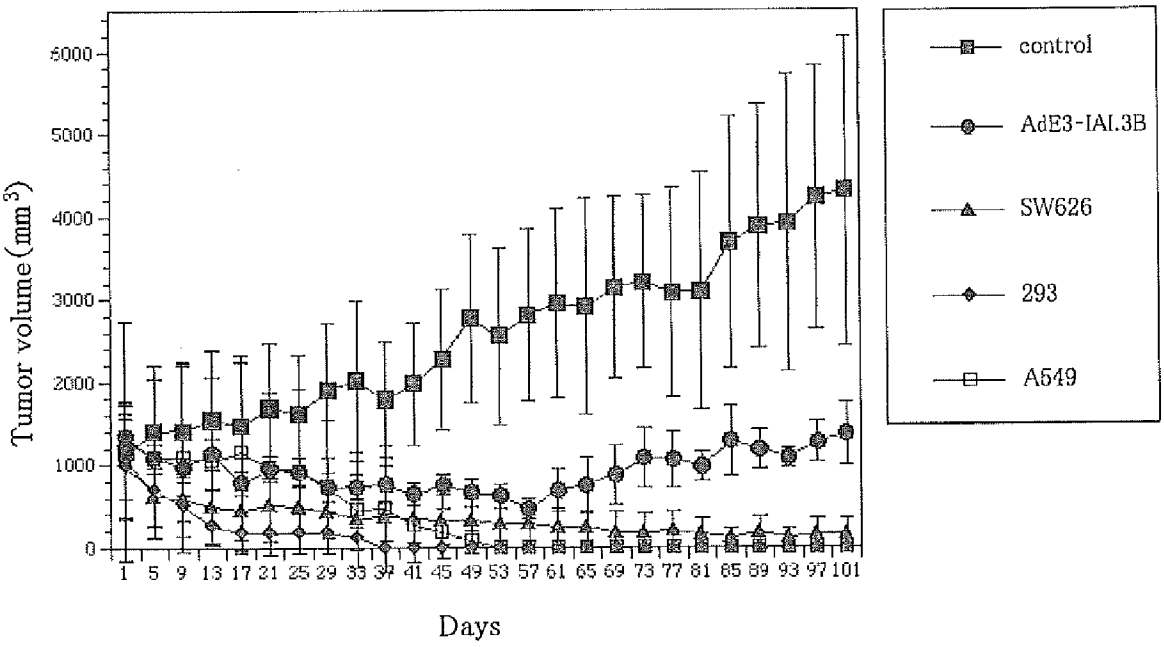

Tumor volume (mm3)
6000
5000
4000
3000
2000
1000
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101
Days
control
AdE3-IAL3B
SW626
293
A549

Fig 6

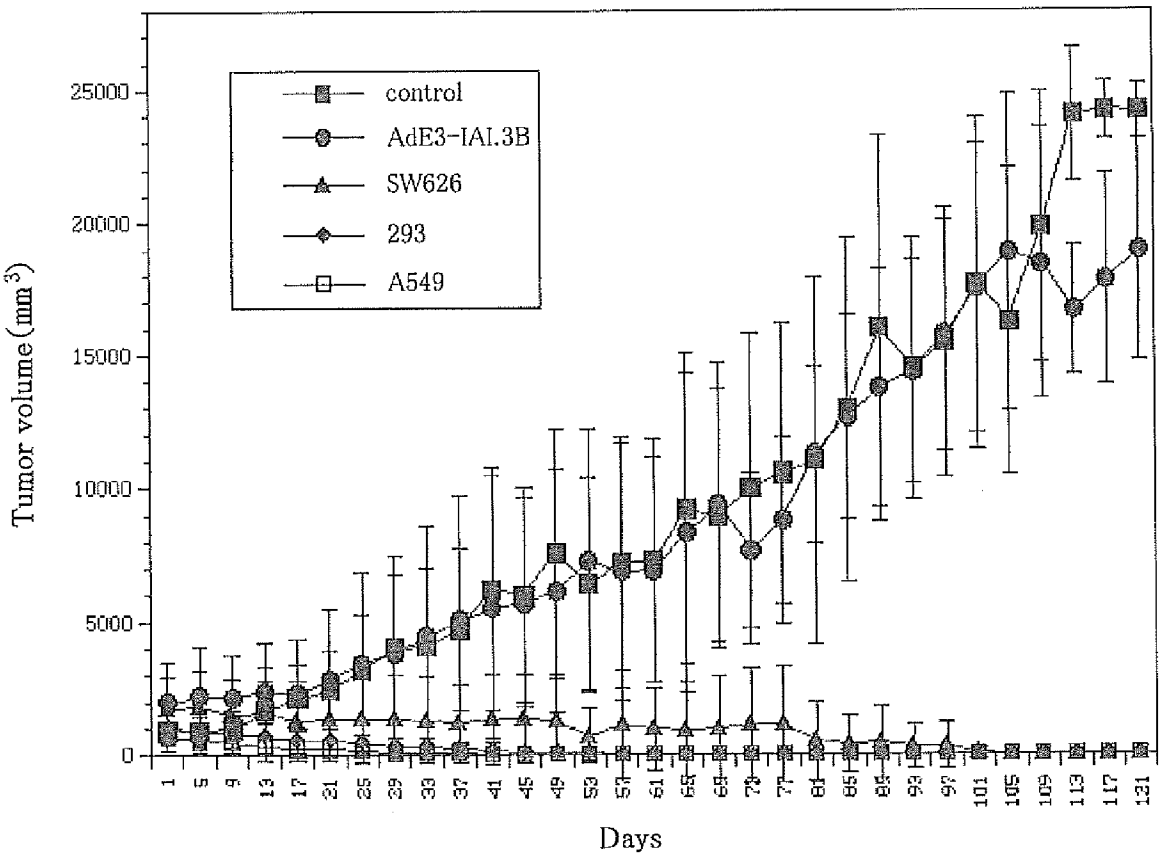

control
AdE3-IAI.3B
SW626
293
A549
Tumor volume (mm3)
25000
20000
15000
10000
5000
0
Days

Fig 7

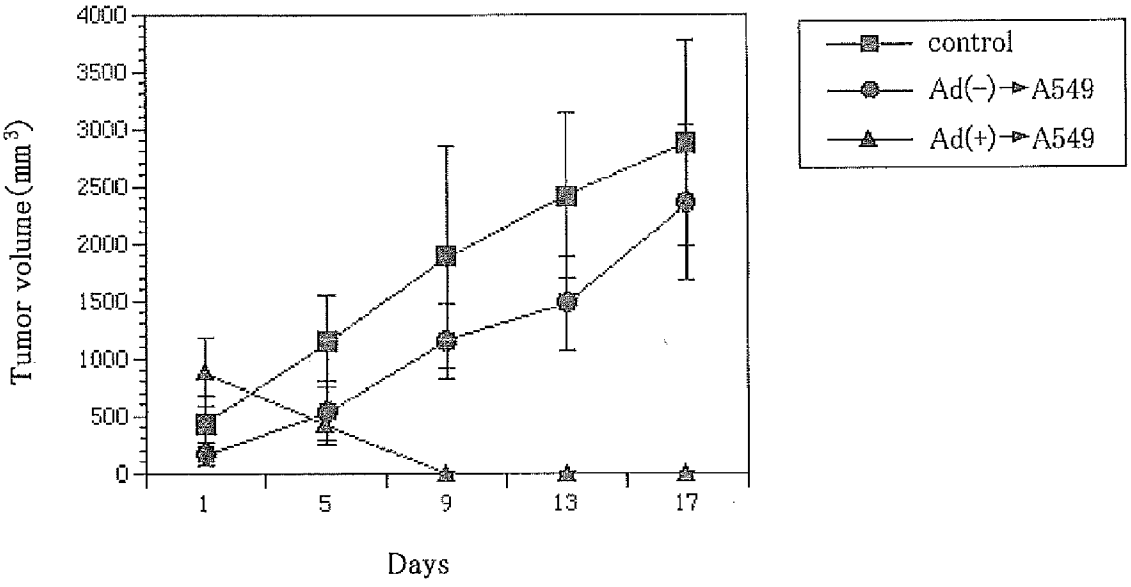

4000
3500
3000
2500
2000
1500
1000
500
0
Tumor volume (mm 3)
1
5
9
13
17
Days
control
Ad(−)→A549
Ad(+)→A549

Fig 8

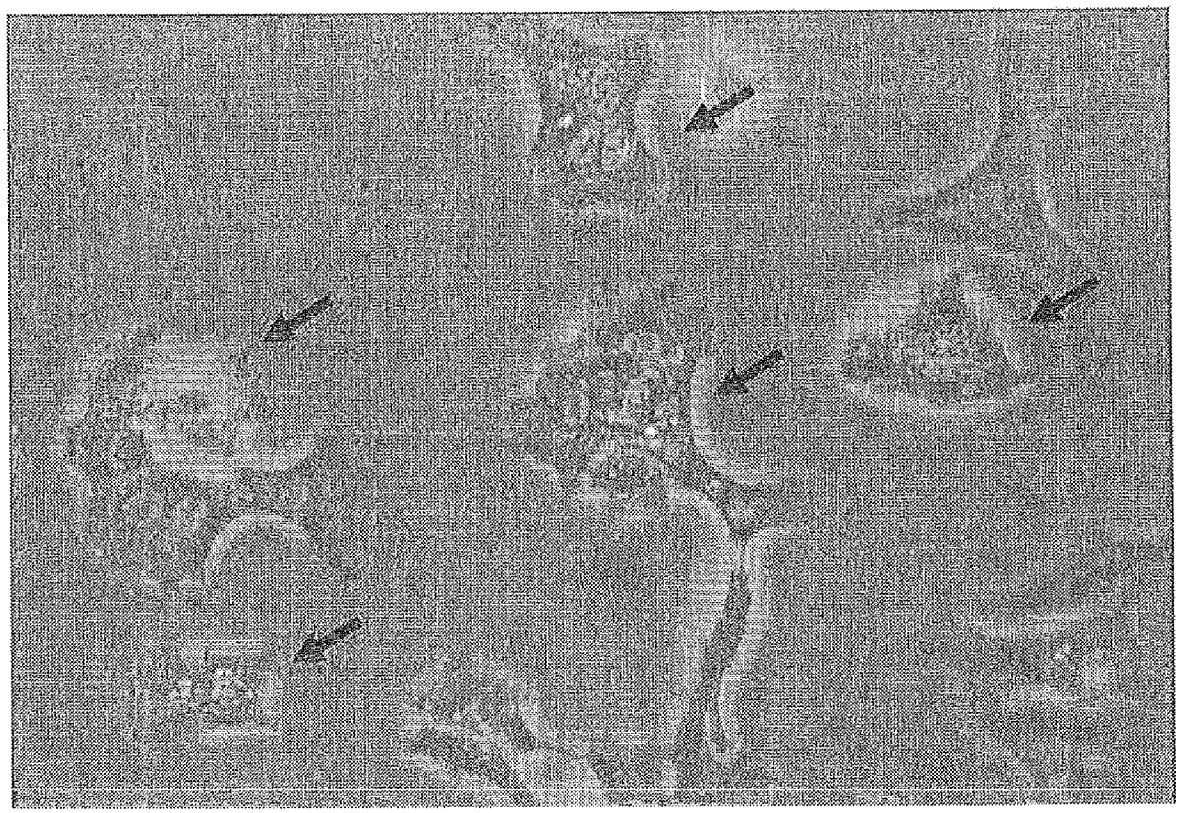


Fig 9

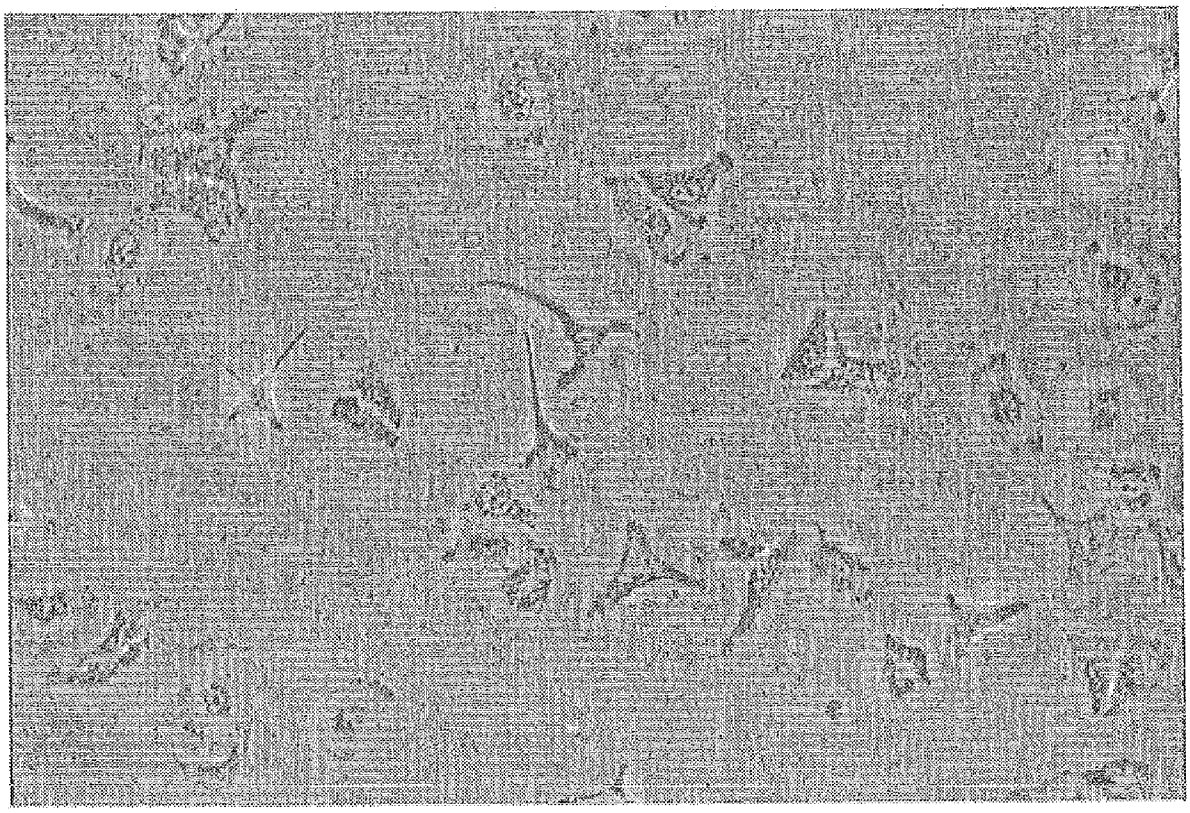


Fig 10

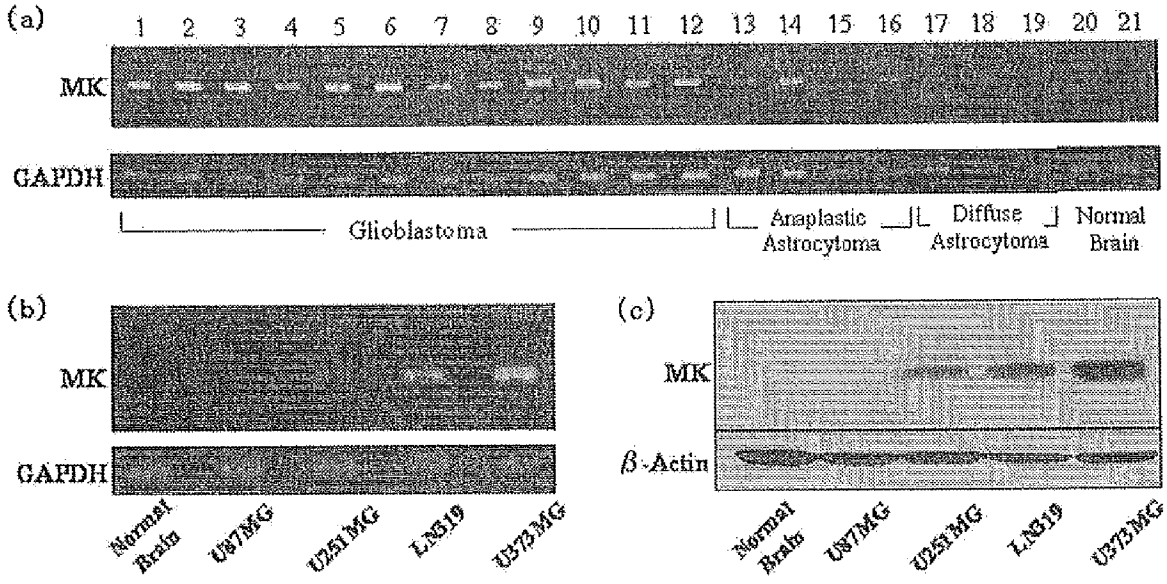

(a)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
MK
GAPDH
Glioblastoma
Anaplastic Astrocytoma
Diffuse Astrocytoma
Normal Brain
(b)
MK
GAPDH
Normal Brain
U87MG
U251MG
LN319
U373MG
(c)
MK
β-Actin
Normal Brain
U87MG
U251MG
LN319
U373MG

Fig 11

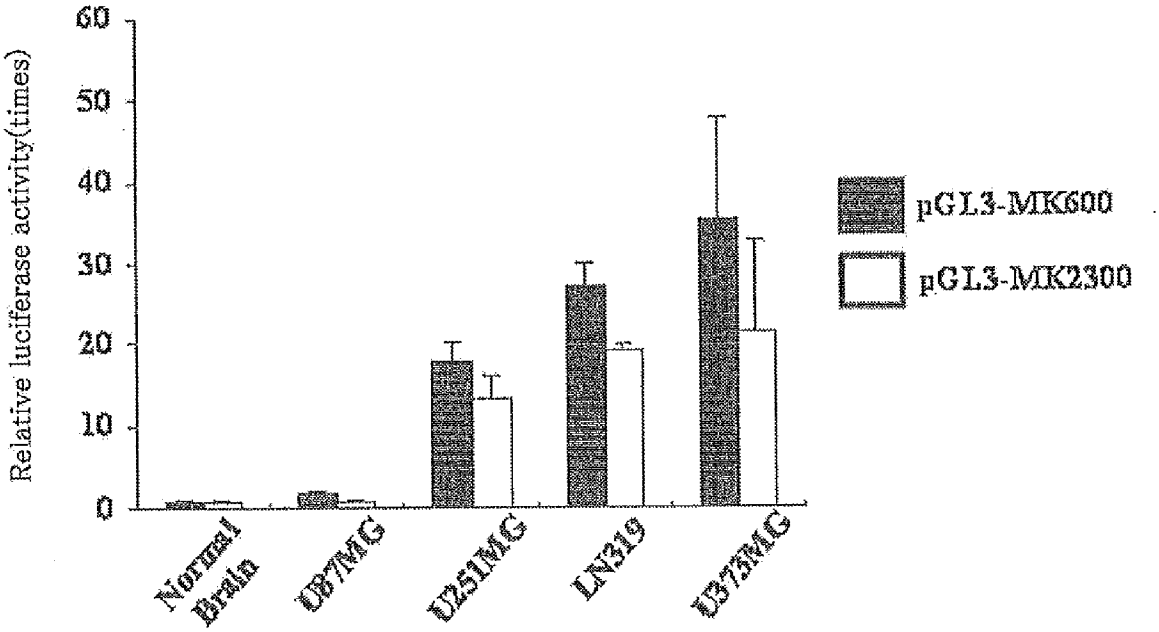
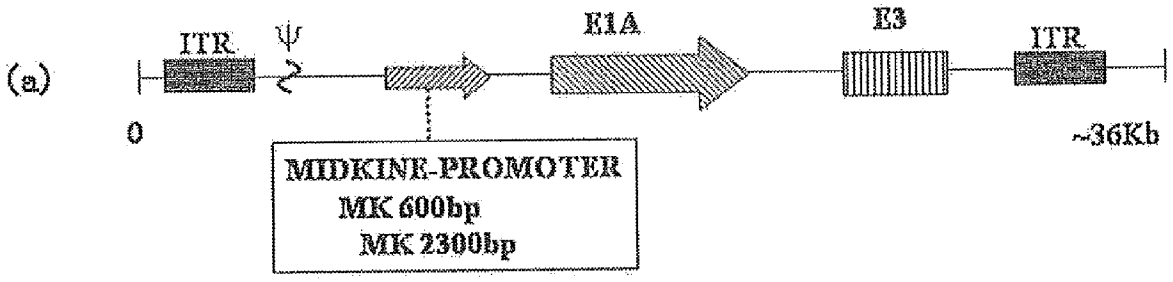

Relative luciferase activity(times)
60
50
40
30
20
10
0
Normal Brain
U87MG
U251MG
LN319
U373MG
pGL3-MK600
pGL3-MK2300

Fig 12


(a)
ITR
Ψ
E1A
E3
ITR
0
~36Kb
MIDKINE-PROMOTER
MK 600bp
MK 2300bp

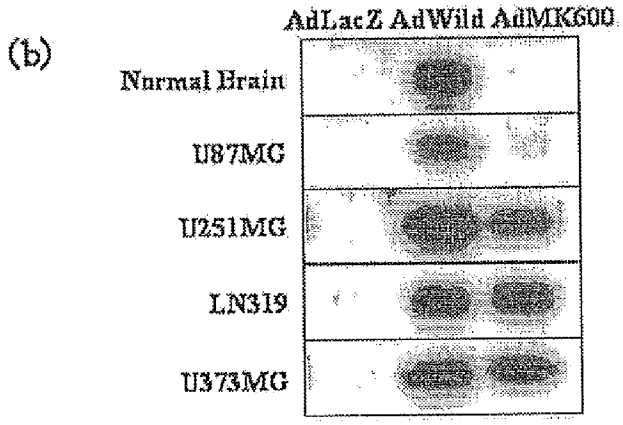

(b)
AdLacZ AdWild AdMK600
Normal Brain
U87MG
U251MG
LN319
U373MG

Fig 13

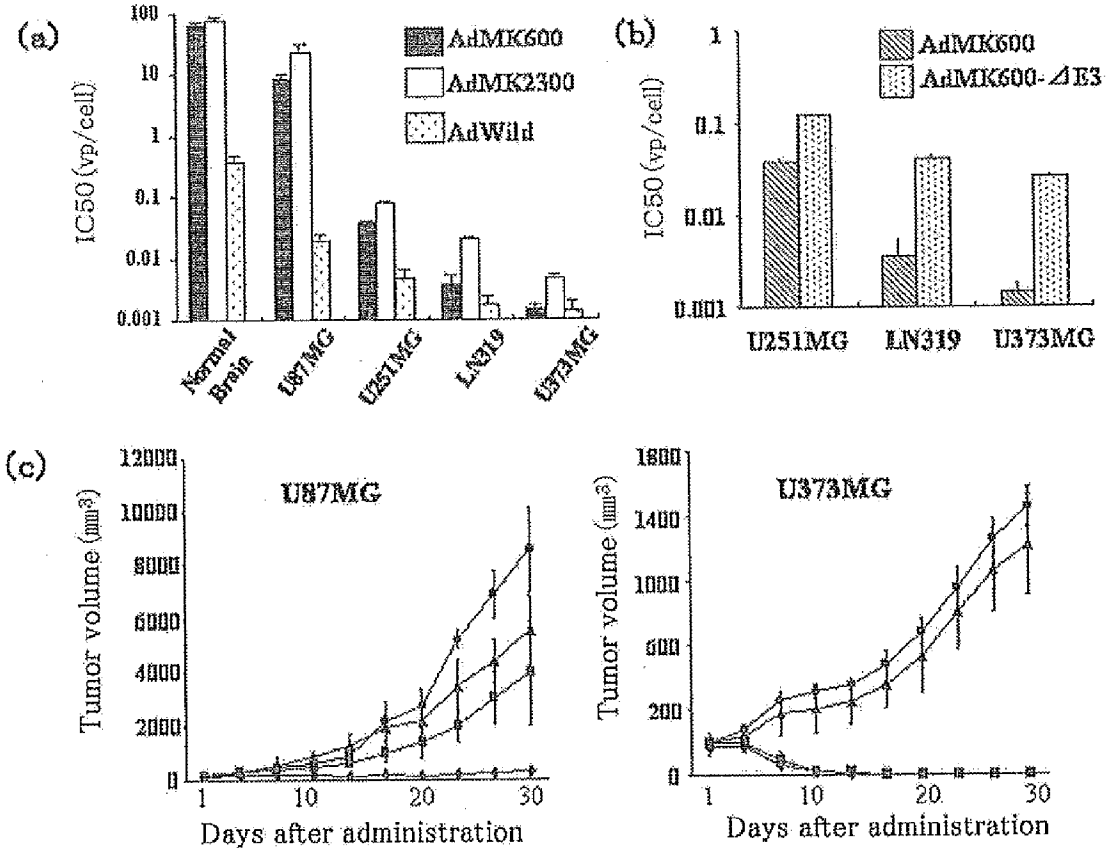

(a)
IC50 (vp/cell)
100
10
1
0.1
0.01
0.001
AdMK600
AdMK2300
AdWild
Normal Brain
U87MG
U251MG
LN319
U373MG
(b)
IC50 (vp/cell)
1
0.1
0.01
0.001
AdMK600
AdMK600-ΔE3
U251MG
LN319
U373MG
(c)
U87MG
Tumor volume (mm³)
12000
10000
8000
6000
4000
2000
0
1
10
20
30
Days after administration
U373MG
Tumor volume (mm³)
1800
1400
1000
600
200
0
1
10
20
30
Days after administration

Fig 14

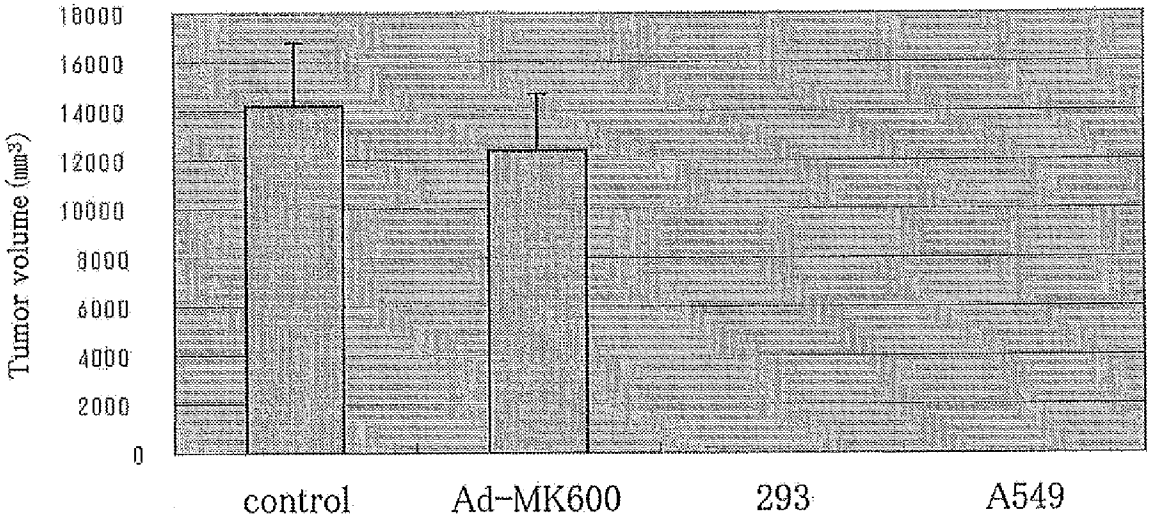

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Tumor volume (mm³)
control
Ad-MK600
293
A549

Fig 15

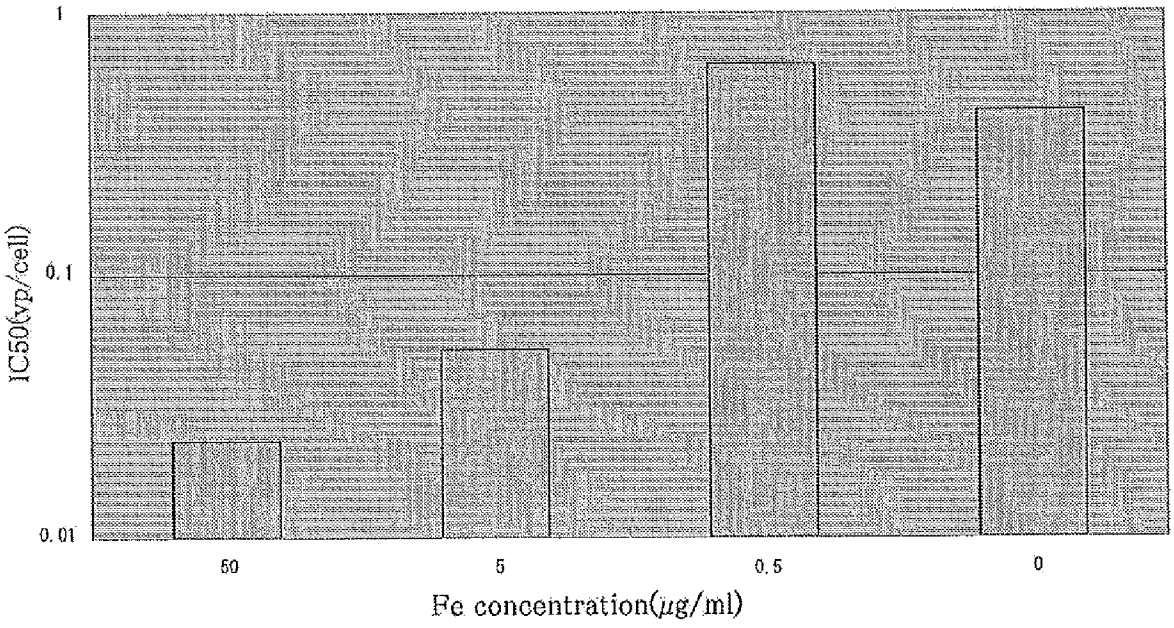


Fig 16

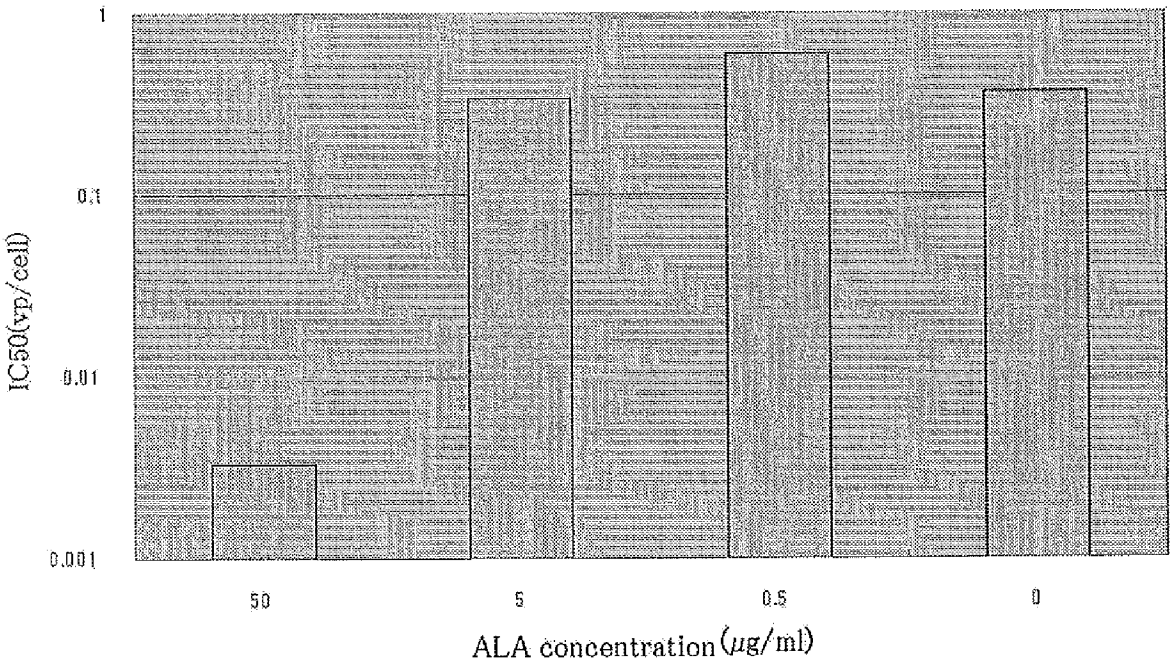
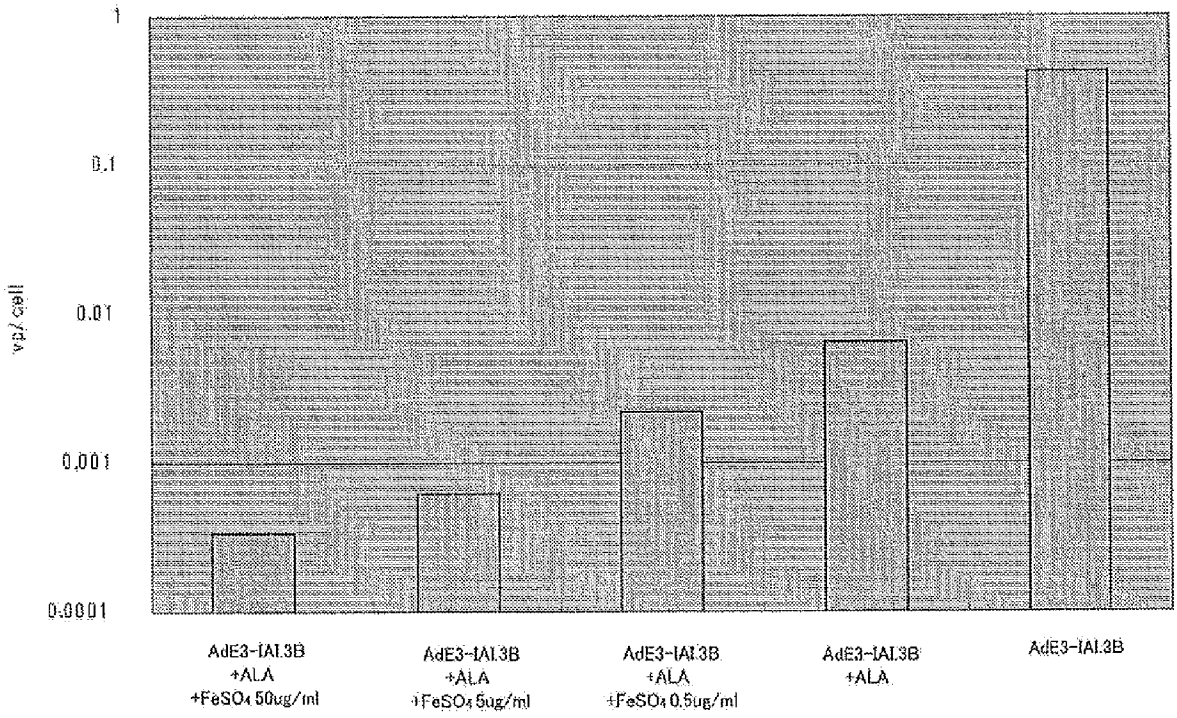


Fig 17


1
0.1
0.01
0.001
0.0001
vp / cell
AdE3-IAI.3B +ALA +FeSO4 50ug/ml
AdE3-IAI.3B +ALA +FeSO4 5ug/ml
AdE3-IAI.3B +ALA +FeSO4 0.5ug/ml
AdE3-IAI.3B +ALA
AdE3-IAI.3B